Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/EP05/000168

International filing date: 10 January 2005 (10.01.2005)

Document type: Certified copy of priority document

Document details: Country/Office: EP
Number: 04077937.3
Filing date: 25 October 2004 (25.10.2004)

Date of receipt at the International Bureau: 16 March 2005 (16.03.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

Europäisches Patentamt | European Patent Office | Office européen des brevets

Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

04077937.3

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

Europäisches Patentamt | European Patent Office | Office européen des brevets

Anmeldung Nr:
Application no.: 04077937.3
Demande no:

Anmeldetag:
Date of filing: 25.10.04
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

Universität Zürich
Rämistrasse 71
8006 Zürich
SUISSE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

A CYANIDE BRIDGED VITAMIN B12-CISPLATIN CONJUGATE

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

A61K47/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL
PL PT RO SE SI SK TR LI

A CYANIDE BRIDGED VITAMIN B_{12} - CISPLATIN CONJUGATE

The present invention relates to a novel compound for the treatment of cancer.

In the field of inorganic medicinal chemistry cisplatin *cis*-$[PtCl_2(NH_3)_2]$ and other derivatives are well established anti-cancer drugs. They are potent therapeutic pharmaceuticals but are also toxic to normal, healthy cells. The relatively large doses administered to a patient causes severe side effects. Enhanced selectivity by targeting cancer cells would be beneficial for the therapeutic index and the life quality of the patient. Site-specific uptake can be achieved by combining biologically active molecules with a chelating moiety for Pt(II). Some attempts have been described but, so far, did not show the expected improvements. A bioactive molecule which merits more attention is cyanocobalamin (vitamin B_{12}) the chemistry of which has been comprehensively reviewed. Fast proliferating cancer cells are so-called high B_{12} consumers. This very high demand makes B_{12} a potential "Trojan horse" for delivering therapeutic agents. Combining B_{12} and Pt(II) compounds has been the subject of earlier studies. *Wilson* et al. described a B_{12} derivative with a diamino chelator covalently attached to the corrin ring yielding a cobinamid-N_2PtCl_2 unit. This conjugate was expected to transport cisplatin into cancer cells by specific surface receptors for the cobalamin-transcobalamin protein complex.

A more direct way would however be the use of native B_{12} as a ligand. Some metal complexes, e.g. $[PdCl_4]^{2-}$ or a mixture of Pt(II) and Pt(IV) species, react quite strongly with cobalamins and demethylates methylcobalamin to aquo- or chlorocobalamin. Interaction of the activated form of cisplatin, $[Pt(NH_3)_2(OH_2)_2]^{2+}$, with alkyl cobalamins is milder. $[Pt(NH_3)_2(OH_2)_2]^{2+}$ binds to N^3 of dimethylbenzimidazole in the backloop, yielding an alkyl-cobalamin base-off form. Binding $[Pt(NH_3)_2(OH_2)_2]^{2+}$ to adenosylcobalamin takes place at the N^7 or N^1 position of the nucleoside ligand.

The inventors aimed at introducing the cis-$[Pt(NH_3)_2]^{2+}$ moiety at the cyanide group. Although cyanide bridges between two metal centers belong to the most common motifs in inorganic chemistry, the cyanide

ligand in vitamin B_{12} has not yet been exploited for derivatization, the only cyanide bridged platinum-B_{12} species described being the adduct of platinum(II)tetracyanide to hydroxycobalamin.

The inventors found out earlier about the coordination of Re(I) and Tc(I) complexes directly to the cyanide in vitamin B_{12} and anticipated that other robust complexes would behave similarly.

In the research that led to the invention they studied the direct interaction of cisplatin with B_{12} to use B_{12} as a ligand and a targeting molecule for Pt(II) the same time (scheme 1).

The B_{12}-cisplatin conjugate 1 can be prepared in good yield by the reaction of B_{12} with the mono-activated form of cisplatin $[PtCl(NH_3)_2(OH_2)]^+$ in water. It could be expected from previous work that $[PtCl(NH_3)_2(OH_2)]^+$ would coordinate preferentially to intermediately released benzimidazole. We found however a straight forward and exclusive coordination to the cyano group in B_{12} resulting in a heterodinuclear complex with [Co]-C≡N-Pt as a central structural unit. The progress of the reaction can be monitored by HPLC. The trace exhibits the formation of one single species in quantitative yield. No intra- or intermolecular trans-metalation to e.g. benzimidazole or cross-linking was observed even after an extended time period. The UV/Vis spectrum, which is about identical to the one of native B_{12}, did not change as would be expected after release of benzimidazole. IR spetroscopy showed a ν_{CN} (st) band at 2199 cm^{-1}, 55 cm^{-1} higher than in vitamin B_{12}. Such shifts to higher energies are characteristic for bridging cyanides. The ^{195}Pt NMR spectra gave a peak at -2340 ppm, which is in the region where cisplatin complexes bound to one purine base with one remaining chloro ligand are usually found. X-ray quality crystals of 1 could be grown. In the asymmetric unit two monocationic B_{12}-platinum conjugates 1 and one trifluoroacetate could be found (Figure 1).

The platinum centres are almost perfectly square planar, with angles ranging from 89° to 92°. The cobalamin unit is barely changed compared with the original B_{12} structure. The bond lengths of the bridging cyanide and from the cobalt to cyanide carbon are not significantly different compared with vitamin B_{12} and Fe-CN-Pt structures. There are two hydrogen bonds formed to the cisplatin

moiety: one between N(29) and Cl(1), the other between N(93) and O(39). The bond angles along the bridge are very close to 180°, unlike in the Fe-CN-Pt case[12] (see Figure 2).

The electrochemical properties of **1** are quite different from vitamin B_{12}. We found a partially reversible reduction wave at -515 mV (0.1 V/s vs $Ag^{+}/AgCl$), which is at a significantly more positive potential than vitamin B_{12} (-670 mV). The increased oxidation power of the Co(III) center can be understood as a consequence of the electron withdrawing properties of the Pt(II) center competing for the electrons in the cyanide bridge.

In the present application reference is made to the following scheme and figures.

Scheme 1. Reaction of cisplatin with vitamin B_{12} (1) and subsequent coordination of 2'-deoxyguanosine to Pt(II) (**2**).

Figure 1. ORTEP presentation of one of the two cations found in the crystal structure of **1**, ellipsoids are drawn with 30% probability.

Figure 2. Close-up of the corrin ring and the cisplatin moiety of figure 1. Selected bond lengths in Å and angles in °: Co(1) - C(90) = 1.879(9), Co(1) - N(81) = 2.043(7), C(90) - N(91) = 1.17(1), N(91) - Pt(1) = 1.953(7), Pt(1) - Cl(1) = 2.300(3), Pt(1) - N(91) = 1.953(7), Pt(1) - N(92) = 2.000(8), Pt(1) - N(93) = 1.982(9), N(29) - Cl(1) = 3.40(1), O(39) - N(93) = 2.96(1), N(91) - C(90) - Co(1) = 174.2(9), C(90) - N(91) - Pt(1) = 173.4(9).

EXAMPLE

Knowledge about some basic biological behavior such as stability in water and human serum or interaction with nuclein bases is crucial to estimate the versatility of such new B_{12}-cisplatin complexes. First, we studied the substitution of the remaining chloride with 2'-deoxyguanosine (2'-dG). Other ligands are also of interest since we anticipate that Pt(II) could mediate the introduction of e.g. a cytotoxic agent in B_{12} by Pt(II) coordination. Complex **1** reacts under mild conditions in aqueous solution with 2'-dG to form quantitatively **2** (**scheme 1**). The reaction is rather slow with an approximate second order rate constant of about 0.2 - 0.3 $M^{-1}s^{-1}$ at 38°C determined under pseudo-first order conditions. NMR analysis of the product indicated

the formation of a 1:1 adduct of 1 and 2'-dG. The downfield part in the ^{1}H NMR spectrum shows the characteristic five signals of B_{12}. None of the signals shifted significantly. The H^8 resonance of the coordinated 2'-dG is split into 3 signals, a main product integrating for 0.65 and two isomers or side products, integrating for 0.1 and 0.25 protons. The ^{1}H signals of the main product confirmed the formation of 2, whereas the identity of the minor peaks is unclear by now. The ^{195}Pt NMR spectrum showed a broad peak at -2475 ppm, in the range where DNA bound cisplatin would be observed.

Compound 1 is stable in water and saline. After one day at room temperature no release of $[PtCl(NH_3)_2(OH_2)]^+$ or $[PtCl(NH_3)_2(NC)]^+$ could be observed confirming high kinetic and thermodynamic stability of 1. The chloride ligand is however slowly exchanged in aqueous solution with water. Measurements with a Cl^- sensitive electrode showed about 10% release of Cl^- after 1 day and about 65% after 4 days. This labile coordination site influences the behavior towards bovine serum albumine. Whereas native B_{12} and 2 show no significant interaction with these proteins in aqueous phosphate buffer, the weakly bound chloride ligand in 1 is exchanged for potential coordinating sites on the protein. After 2 and 6 days, 34% and 47% respectively of 1 did associate with the proteins. Protein binding occurs most likely by chloride exchange on the platinum with competing coordinating sites in the side chains of amino acids.

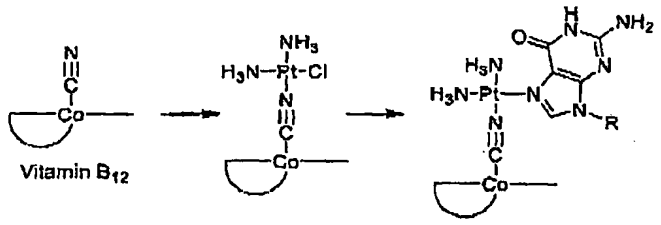
Compound 2 can be considered as a model for a DNA mono-bound cisplatin analog. Since 2 shows only very weak binding to bovine serum albumin, the formation of a stable conjugate is confirmed. Stirring an aqueous solution of 2 with a 10-fold excess of imidazole for one day did not show significant release of dG, $[PtCl(OH_2)(NH_3)_2]^+$ or $[PtCl(NC)(NH_3)_2]$. Obviously, one can not expect from this chemical behaviour that cisplatin is simply released from 1 to start its well known interaction with DNA. However, one must consider that cyanide (and probably also $[PtCl(NC)(NH_3)_2]$) could be cleaved from vitamin B_{12} by reductive elimination in adenosyl transferase or that Pt(II) binds as an antagonist irreversibly in the active site of a B_{12} transporter or a B_{12} dependent enzyme.

CLAIM

Cyanide bridged vitamin B_{12}-cisplatin conjugate as described in the description.

ABSTRACT

The invention relates to $[PtCl(OH_2)(NH_3)_2]^+$, the mono-activated form of cisplatin, coordinates to the cyano ligand of vitamin B_{12} (cyano cobalamin) to form a Co-C≡N-Pt bridged conjugate (**1**). The compound is stable in aqueous solution and has been characterized by x-ray crystallography. The remaining chloride is labile and can be exchanged by stronger coordinating ligands such as 2'-deoxyguanosine to yield stable B_{12}-nucleoside conjugates.



Scheme 1

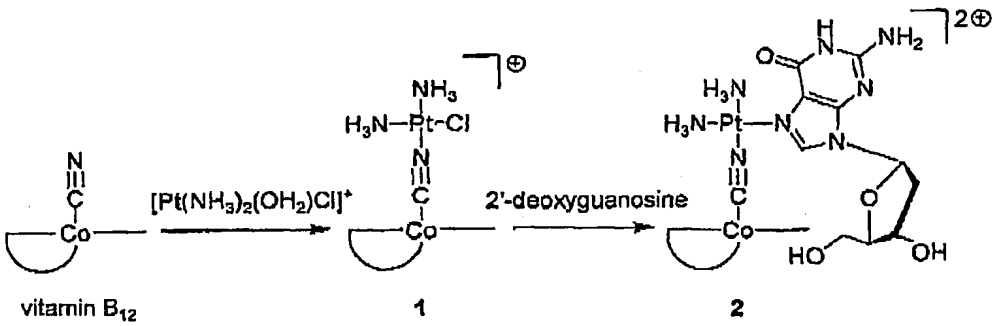
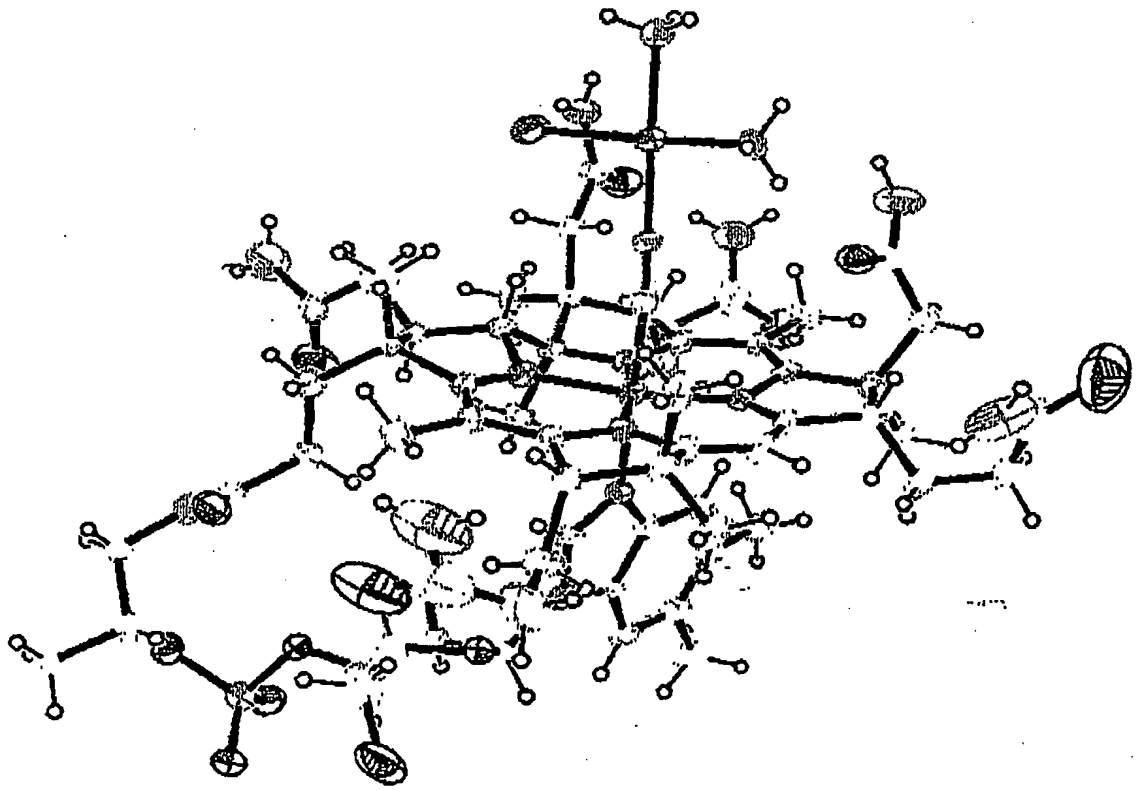


Fig. 1



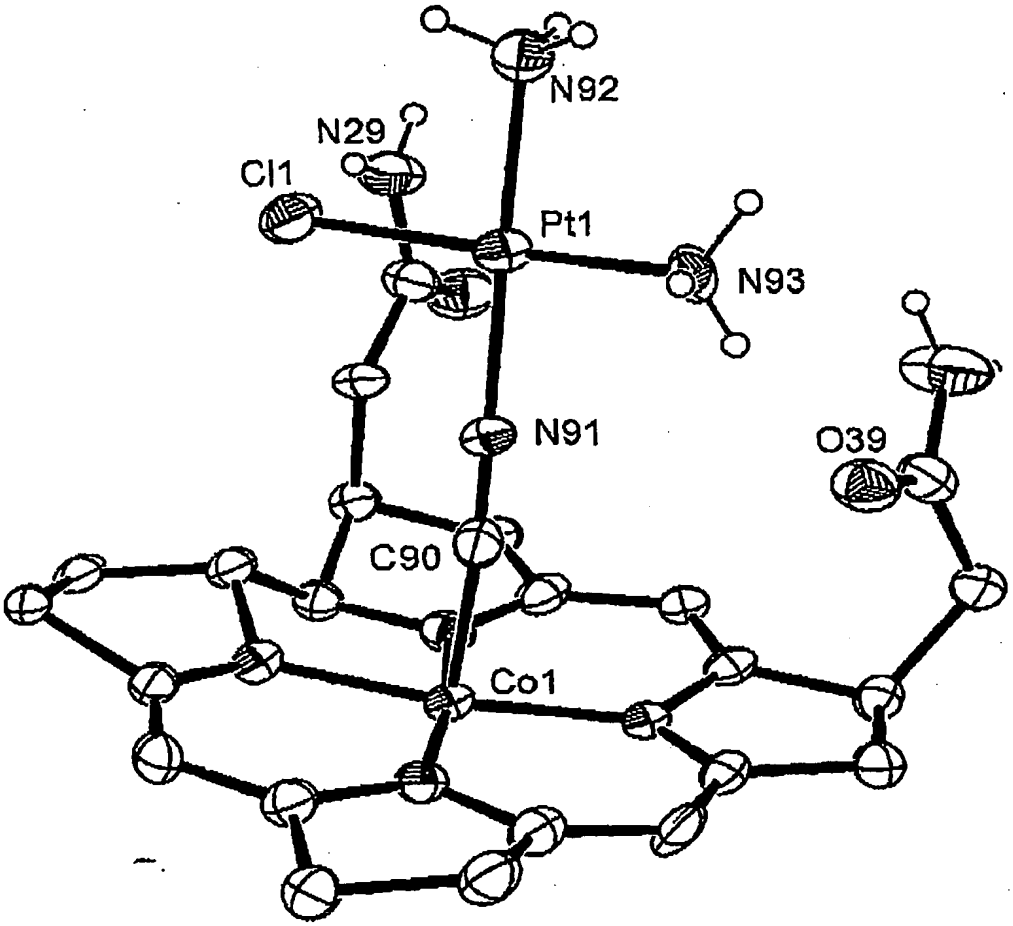


Fig. 2